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December 26, 2001
Date

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Kakefuda et al. Group Art Unit:

Serial No. : 09/893,033 Examiner:

Filed : June 27, 2001

For : CYANOBACTERIAL NUCLEIC ACID FRAGMENTS ENCODING PROTEINS USEFUL FOR...

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified application as follows:

IN THE SPECIFICATION

Please enter the following changes:

Page 14, paragraph beginning on line 2:

The protein phytoene desaturase (PDS, encoded by the gene *pds*) is the target of a number of commercially available bleaching herbicides. The simple cyanobacterial genetic system, *Synechocystis*, was used to generate and select mutant forms of *pds* resistant to bleaching herbicide 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. (BASF (Previously American Cyanamid Company), Princeton, New Jersey.)

Page 14, the paragraph beginning on line 17:

The present invention provides a method to isolate and select mutants resistant to 4'-fluoro-6-[(alpha, alpha, alpha,-trifluoro-m-tolyl)oxy]-picolinamide. Two types of mutants may be isolated: spontaneously produced mutants or chemically induced mutants.

Page 15, Paragraph beginning on line 3.

For isolating chemically induced mutants, ethyl methanesulfone (EMS) may be used. *Synechocystis* cell cultures were treated with EMS at a concentration which gives a 99% killing rate, followed by growth on selection plates. 100 ~200 ml samples of logarithmic liquid culture were harvested and treated with EMS. The reaction was stopped by addition of sodium

thiosulfate, to a final concentration of 5%, to quench excessive EMS. Cells were then collected and washed twice with BG-11. After an overnight recovery in fresh BG-11 medium, cells were plated on solid BG-11 medium containing 1 μM 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

Page 23, paragraph beginning on line 8:

Synechocystis DNA was prepared using the Qiagen Dneasy Plabt Mini Kit (Qiagen, Valencia, California) following NaI pretreatment and digestion with lysozyme as describes in Williams (1988). For manipulation of DNA in *E. Coli*, standard recombination procedures were followed.

Page 16, paragraph beginning on line 3:

In this particular experiment zones of inhibition for the wild type *Synechocystis* cells were observed at the two higher 4'fluoro-6-[(alpha, alpha, alpha,-trifluoro-m-tolyl)oxy]-picolinamide application rates (5 X 10^{-10} mol and 5 X10^{-9} mol) with a diameter of 20 and 38 mm, respectively. However, zones of inhibition were only observed with 4 of the 6 mutants at the highest rate of 4'fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, results with degree of resistance in the following order: 7-3/11F(0) = 7-4/12F(0) > 5-1/12E(8) > 7-3/12F(12) > 5-1/12F(18) > WT(38) (size of zone in mm in parentheses).

Page 25, Paragraph beginning on line 21:

(1) **Lead compounds identification:** This can be done in a reasonably high through put manner using either the paper disc assay on solid BG-11 agar plate or 96-well microtiter plate as described in Section A and Example 1.

(2) **Generation and isolation of resistant mutant(s):** *Synechocystis* mutant(s) resistant to compound of interest can be generated chemically by treating cultures of *Synechocystis* with chemical mutagens (*e.g.* EMS). Procedures for performing such experiment are provided in Examples 2 & 3.

(3) **Isolation of genomic DNA from resistant cell lines:** Genomic DNA can be prepared from cultures of *Synechocystis* resistance cell lines using commercial kits (e.g. Qiagen Dneasy Plant Kit) as described in Section B.

(4) **Primer design and PCR amplification of gene fragments from Synechocystis:** Primer pairs for amplification of overlapping DNA fragments from *Synechocystis* can be designed with the assistance of a commercial software package (*e.g.* Vector NTI from InforMax, North Bethesda, MD). Large-scale synthesis of primers can be done by a commercial vendor (e.g. Sigma-Genosys, The Woodlands, TX) in 96-well format. PCR amplification of ~1800 2-kb fragments (*again, the size of the fragment, thus the total number of primers may be altered for easy PCR amplification and HTP manipulation*) can be performed using genomic DNA prepared from mutant cell cultures as template following standard laboratory procedures, as explained in Section B.

(5) **High Through Put genetic transformation and target site gene identification:** Procedures for HTP genetic transformation and functional complementation assays have been described in Section B. Gene(s) conferring herbicide resistance can then be identified based on the ability of its PCR products to confer herbicide resistance to wild type cells upon transformation.